THE INFLUENCE OF THE TANNIN CONTENT OF THE HOST PLANT ON ENDOTHIA PARASITICA AND RELATED SPECIES[1]

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The differences between species, genera, and families by which certain groups of plants are resistant to certain parasites while others are more or less susceptible are very generally recognized by all botanists. *Actinomyces scabies* attacks potatoes, turnips, beets, carrots, and parsnips, representing four different families. *Pseudomonas tumefaciens* attacks a much larger range of host plants. Many familiar parasitic fungi and bacteria are restricted to certain families, to a few genera, or even to a single genus; others have similar restrictions as regards species; and still others are restricted to the races within the species. The same law will apply to the insects which cause the peculiar physiological or pathological structures known as galls. *Cecidomyia pilulae* occurs on a very large number of oaks, *Amphibolips confluentus* on four species, while the very common *Andricus seminator* occurs on but one.

Individual plants which are more or less immune to destructive parasitic organisms have attracted the attention of the plant pathologists and plant breeders and have been the starting point for long series of selection experiments resulting in resistant varieties which are of great value to the practical agriculturists. Many theoretical explanations of the resistance or susceptibility of related varieties or species have been offered by botanists who should know better, but very few facts of real value have been collected.

MARSHALL-WARD'S studies indicated that the histological characters were of but little if any importance in aiding the plant to resist its parasitic enemies. He finally says: "Infection and resistance to infection depend on the power of the fungus protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and reciprocally, on that of the protoplasm of

[1] A more complete discussion of the experiments given in this paper will appear in some of the publications of the New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

the cells of the host to form auto-bodies which destroy such enzymes or toxins, to excrete chemotactic substances which expel or attract the fungus protoplasm."[2]

The little work that has been done indicates that these problems involve both plant pathology and plant physiology, and that the plant pathologist must give more and more attention to the fundamental problems in plant physiology.

Some years ago the senior author and Dr. J. J. TAUBENHAUS conducted a series of experiments on the relation of parasitic fungi to the cell contents of the host plants.[3] The primary object of this work was to determine to what extent tannin might be a factor in enabling the host plant to resist parasitic fungi. Although the fungi which attack fruits were used for most of these experiments, some attention was given to *Endothia parasitica*. This organism gave a good growth of mycelium and scant spore formation when grown on an agar medium containing 0.6 per cent tannin, but a less toleration to tannin when grown on a liquid medium. It was also evident that its toleration of tannin was somewhat dependent on the character of the food supply.

A little later, CLINTON[4] made similar experiments with *Endothia*, using commercial tannin (M. C. E. Brand, U.S.P.) in amounts varying from 0.2 per cent to 14 per cent. All cultures grew in media containing as high as 4 per cent tannin; about one-half of the cultures of *E. gyrosa* grew on media containing 8 per cent tannin, but all failed to grow on cultures containing more than 12 per cent. *E. parasitica* was a little more tolerant to tannin than the other species used. CLINTON used a potato agar, and therefore his results are not comparable to those obtained by COOK and TAUBENHAUS, who used synthetic media.

Although the results of all of these studies indicate that tannin is in a measure toxic to fungi, the report of the chemist of the

[2] MARSHALL-WARD, H., Recent researches on parasitic fungi. Ann. Botany **19**:1–54. 1905.

[3] COOK, MEL. T., and TAUBENHAUS, J. J., Relation of parasitic fungi to the cell contents of the host plant. 1. Toxicity of tannin. Delaware Agric. Exp. Sta. Bull. 91. pp. 77. *figs. 43*. 1911.

[4] CLINTON, G. P., Chestnut bark disease, *Endothia gyrosa* var. *parasitica* (Murr) Clint. Ann. Report Conn. State Agric. Exp. Sta. 1912. pp. 359–453. *pls. 21–28*. 1913.

Pennsylvania chestnut tree blight disease commission indicated that the tannin content of the diseased bark was higher than that of the healthy bark. This report appears to contradict the idea that tannin in the bark is toxic to the fungus, otherwise the fungus would be destroyed by the tannin. However, this point was also investigated by KERR, who says "the increment of tannin is only apparent and does not really occur. We have found all decayed wood and bark give higher tannin contents, no matter what causes the decay. It simply means that other constituents have decomposed and disappeared, while the tannin remains practically stable."

It is very evident to the writers that commercial tannin is a very uncertain substance, as packages of tannin from the same manufacturers and supposed to be the same were found to give different results when used in cultures. It was also evident that ordinary methods of determining tannin are unsatisfactory. Therefore, KERR, a well known technological chemist of Lynchburg, Virginia, who has devoted considerable attention to the study of tannin, was asked to cooperate in this work. He furnished us with three extracts ("1–X," "2–X," "3–X," and "A") which are described in connection with the experiments. In addition to these extracts we also used commercial tannin (MERCK) for comparison.

Source of cultures

Cultures of various American species of *Endothia*, as well as foreign strains of some of the species, were obtained from various laboratories. In addition, some strains of *E. parasitica* were isolated in our own laboratory. We have indicated these by the names and the serial numbers used by the laboratories when they came to us, except in the case of *E. parasitica*. In this instance we have uniformly referred to this fungus as *E. parasitica*, without regard to whether it was considered a species or a sub-species at the source of supply.

The use of the specific names of *E. gyrosa* and *E. radicalis* varies in different laboratories, according to SHEAR and STEVENS.[5] In the light of this paper it appears that the fungi from CLINTON

[5] SHEAR, C. L., and STEVENS, F. E., Cultural characters of the chestnut blight fungus and its near relatives. U.S. Dept. Agric. Bur. Pl. Ind. Circ. 131. pp. 18. 1913.

labeled *E. gyrosa* and that from STEVENS called *E. radicalis* are in reality identical. Our culture work also leads us to the same conclusion. Therefore, in order to avoid confusion, we will use the name *E. radicalis*, but will in such case indicate the origin of our original culture.

A careful study of our cultures indicates that we have only three distinct species: *Endothia parasitica* (American and Chinese races), *E. radicalis* (*E. gyrosa* and *E. virginiana*), and *E. radicalis mississippiensis*.

Several months were devoted to preliminary work to determine the most desirable medium, best methods for mixing the tannin into the medium, and for perfecting the technique of the work. The formula for the most satisfactory medium, the one which was used in all our work, is as follows: water 1000 cc., glucose 20 grains, peptone 10 grains, potassium phosphate (monobasic) 0.25 grain, magnesium sulphate 0.25 grain. A given series of cultures was always made from the same lot of medium, treated with the same extract, inoculated in the same manner, and kept under exactly the same conditions.

The difficulties arising from the use of tannin in a medium containing proteid were not overcome. The first difficulty encountered is the fact that commercial tannin (MERCK) is an unstable and variable substance. According to FISCHER (Ber. Deutsch. Chem. Gesells. **36**:3252. 1913), tannin is an anhydrous glucoside of gallic acid. This relationship makes it easily convertible by hydrolysis into gallic acid and related substances. *It is therefore entirely possible that no sterile culture medium can be prepared which contains all the tannin unchanged.*

The usual statement that tannin in contact with proteid forms an insoluble precipitate has not been borne out by our work. Indeed, comparatively large quantities of tannin may be added to the agar formula which we used without changing perceptibly either the tannin or the proteid, so far at least as we were able to determine.

The experiments were conducted with two lots of MERCK'S tannin. The first of these was already in stock at the time the work was undertaken. By using a 10 per cent solution of this tannin, as much as 2 per cent of tannic acid could be added to the

agar without changing the composition of either. However, to accomplish this it was necessary to allow the tannin solution to run slowly from a pipette into the melted agar while the latter was constantly agitated. If the tannin was added too rapidly, or the agitation of the agar was insufficient, more or less coagulation resulted. With a 20 per cent aqueous solution of tannic acid, less than half this amount (0.8 per cent) could be added without coagulation. Moreover, even a very small amount of tannic acid in its solid form would cause coagulation in the agar. Another lot of MERCK'S tannin was of such character that only about half as much tannic acid could be added to the agar without change.

When first placed in the agar, the tannic acid caused a milky appearance, which disappeared on sterilization. Where high percentages of tannin were used, the agar upon sterilization showed a tendency to become viscid (about 0.8–2 per cent) or even liquid (about 2–2.5 per cent). The transition between viscosity and liquefaction is gradual in such a series as we used, where each member differed from the next by 0.2 per cent of tannic acid. In no case was the distinct curd which various investigators have described to be observed. In agar with 3 per cent of tannic acid the entire mass of medium becomes a clear liquid with a thin film of solid matter on one side of the test tube if set for a slant, or in the bottom if set upright. This solid material gives the same reaction both to Millon's proteid test and to the ammonium molybdate test for tannic acid as does the solid agar of the lower members of the series. Similar results were obtained by testing the liquid portion of the medium. Evidently, the explanation of this liquefaction is to be found in some other direction than the chemical interaction of tannin and proteid.

If the agar medium used is titrated to various degrees of acidity and a series of such tubes sterilized, it is found that the agar ranges from solid through viscid to liquid; that is, the same phenomenon can be induced by acidulating the medium as by the addition of the tannic acid. In each case a more careful test of the nature of the proteid substance in the liquid from the acidulated agar shows that proteid digestion has progressed so far that the power of solidification has been lost.

These considerations naturally raise the question of the acidity of the culture medium containing tannic acid. Our tests showed that a 3 per cent aqueous solution of tannic acid is about +65 Fuller's scale. This is considerably higher than is indicated by CLINTON (Report Conn. Agric. Exp. Sta. 1912. p. 432). However, as CLINTON used a vegetable (potato agar) medium, while we used a synthetic medium, the results are in no wise comparable, as no account is taken by CLINTON of the effects on the tannic acid of the various organic constituents of the medium to which he added it.

Gallic acid was similarly tested, but failed to show any coagulating effect either on the agar medium or on its constituents.

The various materials furnished by KERR behave in much the same way toward agar as does commercial tannin. His purer tannin extracts, however, do not liquefy the agar at as low percentages as does commercial tannin. Those extracts from which the coloring matter had not been removed had a more pronounced action on the culture medium than even the second lot of MERCK'S tannin. The original acidity of the agar and the quantity and nature of the impurities which may be present in the tannin appear to modify to a great extent the chemical activities upon the admixture of the two substances in completing the culture medium.

To test tannin fermentation

To test the ability of the fungi to live in pure solutions of tannin and related substances, two experiments were made with varying strength of aqueous solutions of these materials. In the first series, the spores were sown on the medium, and after germination cubes about 5 mm. in size were transferred to the tannin solution. In these tests *E. parasitica* and the European strain of *E. radicalis* were used.

The first test included MERCK'S tannin and KERR'S extracts "A" and "1–X," of 2.05 per cent and 5 per cent solution. The strength of the solution appeared to have less effect on the fungus than did the nature of the material. The two species of fungi showed no more difference in quantity and vigor of growth than would be expected for two strains of the same species.

On tannin neither species grew even as much as might have been expected from the nutriment stored in the agar block.

On extract "A" a fair growth was made, using up the food material stored in the original agar block and forming masses of mycelium about 2 cm. across and producing abundant pycnospores.

On extract "1–X" an abundant growth was secured, entirely filling the liquid in the flask with a thick growth of mycelium which rose above the liquid and produced abundant pycnospores.

The second series was made up in strength of 0.2, 0.4, 0.6, 0.8, and 1 per cent tannic and gallic acid. These were sown with spores of the same fungi used before. In no case was growth made, although check sowing on agar showed the spores to be viable.

Endothia on tannin

GROWTH OF ENDOTHIA ON COMMERCIAL TANNIN (MERCK)

The cultures used for this series were made to contain 0.1 per cent, 0.2 per cent, 0.4 per cent, and by intervals of 0.2 per cent up to 2.4 per cent of tannin. In this series of experiments the agar remained firm, except in one or two of the cultures containing the highest percentages of tannin, in which there was a slight tendency to a semifluid condition. No cultures showed sufficient proteid digestion to allow the formation of a liquid. The following strains of *Endothia* were used: *E. parasitica* (STEVENS no. 1158), a Chinese strain from the Bureau of Plant Industry,[6] and a strain designated "P.P." of our own isolation from Prospect Park (Brooklyn) material; *E. radicalis* (METCALF no. A, STEVENS no. 2391, a European strain secured from ANDERSON, and CLINTON'S *E. gyrosa* no. 7677); and *E. radicalis mississippiensis* (STEVENS nos. 1196 and 3443).

Endothia parasitica (American strain) gave a good growth of aerial mycelium, varying in amount in direct ratio to the percentage of tannin used. Poor growth on check until the end of the second week. Yellow color in mycelium at end of first week and discoloration of the agar during the second week. Cultures originally containing 0.2 gm. tannin (2 per cent) were sent to KERR,

[6] This culture was made from material sent directly from China to the Bureau of Plant Industry.

who was unable to detect any positive trace of tannin, but found a trace of gallic acid not exceeding 0.002 gm. This appears to to indicate that the fungus can use the tannin as a food. Pycnospores appeared on the check at the end of the sixth week, on 0.1 per cent tannin at the end of the seventh, on 1.4 per cent tannin at the end of the eighth, and ultimately on the 4 per cent tannin.

E. parasitica (Chinese strain) grew about the same as the American strain except that the growth was 5 days earlier and a lighter gray color. Pycnospores appeared during the fifth week on 2.4 per cent tannin and ultimately on the entire series.

It is rather remarkable that the Chinese strain is more tolerant to tannin than the American strain, and it raises the question whether this resistance is due to origin, age, or modification of the fungus since its first introduction into America, or some other cause.

E. radicalis (European) was more resistant than the American strain. Pycnospores were produced on 1.2 per cent tannin after 8 weeks' growth.

E. radicalis (American) gave an abundant growth of aerial mycelium, varying in color from yellow in the lower percentage of tannin to ashen in the higher. The presence of 0.2 per cent tannin was stimulating, but 0.8 per cent retarded the growth. No pycnospores were produced.

E. radicalis (CLINTON'S *E. gyrosa* no. 7677) grew well, but no pycnospores were found. The tannin had an inhibiting influence.

E. radicalis mississippiensis grew well, but apparently was not able to use the tannin as food. No pycnospores were produced. The two cultures (nos. 2443 and 1196) used were very resistant to tannin, the former making the more vigorous growth.

From this series of cultures it appears that tannin (MERCK) affects various species of the genus *Endothia* quite differently. *E. parasitica* may for a time be retarded in its growth, but subsequently it feeds on the tannin, using the entire supply in the cultures tested. At the other extreme is *E. radicalis mississippiensis*, which appears to be entirely unaffected by tannin, and does not feed upon this substance. The cultures labeled *E. radicalis* (and CLINTON'S *E. gyrosa* no. 7677) are inhibited by the action of tannin. This is

true of the American strains to a greater extent than of the European.

GROWTH ON KERR'S EXTRACTS

Extract "1–X" is described by KERR as the water soluble tannin of the chestnut bark. It is insoluble in alcohol and in similar solvents. This occurs in quantities of 3–5 per cent in the bark. The sample used was between 95 and 100 per cent pure. The quantity available would not allow as extensive a series of cultures as were used for commercial tannin products. Accordingly, the percentages used were 1, 1.2, 1.6, 2, and 2.4 per cent. The agar remained firm in all cases. Inoculations were made with *E. parasitica* (STEVENS no. 1158), *E. radicalis*, and *E. gyrosa* (CLINTON'S no. 7674).

E. parasitica (American) gave an abundant aerial growth during the third week and of pycnospores during the fifth week. The great growth of pycnospores was on 2 per cent, which is about the normal amount in the bark. This extract is stimulating in normal and subnormal amounts.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) gave a growth similar to *E. parasitica*. At the end of the first week the most normal growth was on 1.2 per cent and the maximum on 2 per cent. Pycnospores appeared in 10 days and were present on all cultures at the end of the fifth week. Finally, it may be said that small quantities of this extract are stimulating, and that higher percentages produce vigorous growth of aerial mycelium and reduced number of pycnospores. It is rather surprising that this extract, which is so near pure tannin, is not as toxic as the commercial tannin.

Extract "2–X" is in all its reactions similar to that designated "1–X," except that it is soluble both in water and in alcohol. Its effect on agar is quite different, however, showing a tendency to digest proteids as do acids, and so render the medium viscous. A series of cultures was prepared containing 1, 1.2, 2, and 2.4 per cent of the extract, and inoculated with *E. parasitica* (STEVENS no. 1158) and *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674). The cultures of the two upper members of the series were quite noticeably viscous.

E. parasitica (American) gave growth on 1–1.6 per cent in one week, on 2 per cent in two weeks, and on 2.4 per cent in four weeks. Pycnospores were produced rather abundantly on 1.6–2.4 per cent at the end of two months.

Endothia radicalis (CLINTON'S *E. gyrosa* no. 7674) gave a good growth on 1.6 per cent in one week, and on 2 per cent three days later. In two weeks the growth was greater than with any other extract used, the maximum on 1 per cent and 1.2 per cent. Pycnidia began to appear on 1.2 per cent in about three weeks and finally on 1 per cent and 1.6 per cent, the maximum on the last percentage.

It is very evident that extract "2–X" is not so favorable for the growth of the fungus as extract "1–X." Both these results are surprising when compared with those obtained with commercial tannin.

Extract "3–X" is the coloring matter of the bark. While this is estimated as tannin in bark analysis, its real nature is unknown. It precipitates gelatin and combines with hide, but does not give the same distinct reactions with metallic salts as do other tannins. The sample used was between 85 and 90 per cent pure. As the quantity available was very small, it was used only in the proportion of 1 and 2 per cent. Both *E. radicalis* (*E. gyrosa* CLINTON no. 7674) and *E. parasitica* (STEVENS no. 1158) were grown on these media.

E. parasitica (American) gave a slight growth on 1 per cent of the extract in one week. This growth increased slowly, but finally became abundant. Germination was retarded for about 10 days on 2 per cent of the extract, and the growth was never so good as on 1 per cent. Pycnospores were found on cultures containing as much as 1 per cent of the extract during the first week, but were never found on the medium containing 2 per cent.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) gave a growth very similar to that of *E. parasitica*, but at the end of two months there were few pycnospores on the 1 per cent extract.

This extract was very toxic; the growth was always unhealthy and the production of pycnospores greatly checked. These results are surprising in that this extract, which is primarily coloring matter

of the bark, which under ordinary methods of analysis is estimated as "tannin," is more toxic than commercial tannin. KERR in commenting on these results, says:

> The action of "3–X" is also surprising, as it is what we term the coloring principle of the bark, the exact nature not having been determined by anyone that I know of. Its action brings out a rather interesting point, and that is that chestnut trees of northern growth, say on a line north of the southern boundary of Pennsylvania, contain very materially less coloring matter than the growth south of it, and, as we all know, the wood in the latitude referred to seems to have been more susceptible to the disease than that further south [letter December 26, 1913].

GROWTH ON COMBINATION OF KERR'S EXTRACTS

Since "1–X," which is a tannin extract, was stimulating, and "3–X," which is primarily coloring materials giving tannin reactions, was toxic, it was decided to combine the two into one extract. The material was made up into a series of cultures containing 0.2, 0.6, 0.8, 1.2, 1.6, 1.8, 2, 2.2, 2.4, 2.6, and 2.8 per cent. Sowings were made with *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674), *E. radicalis mississippiensis* (STEVENS no. 2424), and both American and Chinese strains of *E. parasitica*.

There was a tendency for the agar containing as much as 1.2 per cent of the extract to become less solid, but even with 2.8 per cent there was no approach to a real liquid condition.

E. parasitica (American) made a fair growth on 0.6 and 0.8 per cent in one week, with a slight growth throughout the series except on 0.2 and 2.2 per cent. The growth increased, but was relatively the same on the different cultures throughout the entire period. During the third week pycnidia appeared on 0.6 per cent, and by the end of the fifth week had developed on all cultures up to 2.2 per cent. In cultures containing more than 2.2 per cent the pycnospores decreased. The growth was always subnormal, but not so pronounced as in some other cultures.

E. parasitica (Chinese) also failed to grow on 0.2 and 2.3 per cent, but made some growth on 0.6, 0.8, and 1.2 per cent during the first week. At the end of the second week the cultures containing 0.6 per cent showed a subnormal growth, and the 2.8 per cent a very slight growth. The growth generally was less than that

of the American strain. Pycnospores were not found on either the check or the cultures containing tannin.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) made a fair growth on 0.2, 0.6, and 0.8 per cent, and a slight growth on the higher percentages. The growth generally increased on all cultures, but was always subnormal; no pycnospores were found.

E. radicalis mississippiensis grew on only the 0.8 per cent extract during the first week. In 10 days there was good growth on both 0.6 and 0.8 per cent, fair growth on 1 and 1.6 per cent and slight growth on 2 and 2.8 per cent. The growth increased on all cultures during two months' observation, and pycnospores were produced in abundance on all cultures up to 2 per cent.

In general it may be said that *E. parasitica* and *E. radicalis* thrive fairly well on cultures containing these extracts, but not so well as on the checks. The American strain of *E. parasitica* is more resistant that the Chinese strain, and *E. radicalis mississippiensis* is the most resistant of any species used.

KERR'S extract "A" is a compound of various forms of tannin and of other more or less related substances. It represents about 9 per cent of the dry weight of the bark. Its composition is as follows: tannin (containing the forms represented as "1–X," "2–X," and "3–X") 60 per cent, fermentable sugars 10 per cent, gallic acid 7 per cent, pentoses and pentosans 8 per cent, water 5 per cent, undetermined 10 per cent. This extract produces a rather advanced proteid digestion, causing the agar to become semi-fluid, and in the higher percentages used a considerable amount of fluid was present. The series included 1, 1.2, 1.6, 2, and 2.4 per cent of the extract. Sowings were made with *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674) and *E. parasitica* (STEVENS no. 1158).

E. parasitica (American) made a slight growth on all cultures during the first week. In 10 days the growth was good on all cultures up to 2 per cent. After that time the growth was slow. At the end of the first month pycnospores began to appear on cultures containing 1 per cent, but did not appear on others. This organism made its best growth on this medium.

E. radicalis (CLINTON'S *E. gyrosa* no. 7674) made a slight growth on cultures up to 1 per cent. The growth was poor throughout

the entire time. A very few pycnospores were produced on the 1 per cent, but not on the others.

This was the most toxic extract used in the entire series of experiments.

In order to test the possibility of preparing from commercial sources a compound similar in its effects to KERR'S extract "A," a tannin compound was made as follows: tannin (MERCK) 60 gm., dextrose 10 gm., gallic acid 7 gm., arabinose 8 gm., total 85 gm. This material was added to the agar in the same manner as was the tannin. The series of cultures prepared were at intervals of 0.2–3.0 per cent. The effect on the proteids was the same for this substance as for tannin. The higher percentages digested almost all proteids so that the medium was a clear liquid. Sowings were made with *E. radicalis mississippiensis* (STEVENS no. 2424), *E. radicalis* (CLINTON'S *E. gyrosa* no. 7674), *E. parasitica* (American, CLINTON'S no. 7675, and the Chinese strain).

It seems scarcely necessary to give a detailed statement of these results. In all cases, except the American strain of *E. parasitica*, pycnidial formation was greatly retarded. Growth was very similar to that obtained with MERCK'S tannin.

Summary

1. Results obtained with commercial tannin are not always comparable to each other or to those obtained from specially prepared extracts, because of variations in chemical composition and the presence of tannin-like substances other than tannic acid. Commercial tannins of the same brand differ in their behavior in culture media, as indicated by the growth of the various species of *Endothia* used in these experiments.

2. Commercial tannin and tannin in the plant are not the same. No extract will be the same as the substances in the plant.

3. Tannin is an anhydrous glucoside of gallic acid and is easily converted by hydrolysis into gallic acid and related substances. It is very doubtful if any culture medium can be prepared containing as much pure, unchanged tannin as was put into it. Therefore, we cannot know the exact percentage of tannin in a culture medium,

but we can put known amounts into those with which we are working.

4. The quantity and form of tannin compounds present in the substratum each exerts an influence on the growth of the fungus; but when the fungus attacks a plant, we have no way of knowing the form of the tannin with which it comes in contact. However, it is quite evident that the tannin of the plant is associated with coloring materials and other substances, some of which are toxic. Furthermore, the fungus may be selective during either a part of or during its entire existence, and send its mycelium into certain tissues containing little or no tannin. *E. parasitica* is especially destructive because it works in the cambium cells, but later in life it pushes through the outer tannin-bearing cells of the bark for the production of its spores.

5. The character of the food supply influences the vigor of the fungus, and therefore its power to resist the toxicity of the tannin and other materials with which it comes in contact. The amount and character both of the food supply and of the tannin and other materials no doubt vary with the seasons and the growing periods of the host plant.

6. In almost every instance, without regard to the form of tannin used or the fungus grown, a high percentage (0.8 per cent or more) of tannin caused a retardation of germination, frequently followed by an abnormal stimulation to growth of aerial mycelium.

7. Species of *Endothia* show a marked response to the presence of tannin and related substances in the culture medium.

8. The species of *Endothia*, and to a certain extent strains of the same species, show a considerable variation in their response to tannin and other substances. (*a*) *E. radicalis mississippiensis* was unaffected by the tannin, but did not use it for food and did not produce pycnospores in cultures containing tannin. (*b*) *E. parasitica* was slightly retarded in its germination and early growth, but later was able to use as much as 2 per cent tannin as food. It was the only species studied that was able to utilize any considerable amount of tannin for food. *The American strains were more resistant to tannin and associated toxic materials than the Chinese strains.*

(*c*) *E. radicalis* (including *E. gyrosa*) was very susceptible to the influences of tannin.

9. Tannin (MERCK) affects the various species of *Endothia* very differently. *E. radicalis* (including *E. gyrosa*) is inhibited; *E. parasitica* is at first retarded and later is able to feed on the tannin; *E. radicalis mississippiensis* is practically unaffected and does not feed on tannin.

10. Analyses of chestnut bark made by KERR show a corresponding tannin reduction in diseased areas, which confirms the culture experiments and makes it possible to state that *E. parasitica* is able to use the tannic acid for food.

11. *E. parasitica* appeared to have its power of pycnidia production stimulated by commercial tannin and the true tannin extracts of chestnut bark, but reduced or inhibited by those extracts of chestnut bark which are composed almost entirely of coloring substances, but which are present in tannin extracts and estimated as tannin.

12. Specially prepared extracts of pure tannin were either stimulating or only slightly toxic when combined with coloring materials and other substances associated with tannins and responding in the same or in a similar manner to tannins. (*a*) KERR'S "1–X" extract has a stimulating effect on *E. radicalis* (*E. gyrosa*) and *E. parasitica*. (*b*) KERR'S "2–X" extract has a tendency to retard *E. radicalis* (*E. gyrosa*), *E. parasitica* (both American and Chinese strains), and *E. radicalis mississippiensis*. (*c*) KERR'S "3–X" extract was extremely toxic to *E. radicalis* (*E. gyrosa*) and *E. parasitica*. (*d*) In *E. radicalis* (*E. gyrosa*) conidia production was at its maximum at 1–1.2 per cent of the extracts designated "1–X," "2–X," and "3–X," while but few if any pycnospores were produced on the other substances used. (*e*) The European strain of *E. radicalis* showed similar results, but the American strain showed a tendency to remain sterile. (*f*) A combination of "1–X" and "3–X" is somewhat toxic, but the toxicity of "3–X" appears to be largely overcome by the stimulating influence of "1–X." *E. radicalis* (*E. gyrosa*) and *E. parasitica* were slightly retarded, and *E. radicalis mississippiensis* was very slightly retarded. The Chinese strain of *E. parasitica* was less resistant than the American

strain. (*g*) KERR'S "A" was most toxic of all compounds on *E. radicalis* (*E. gyrosa*) and *E. parasitica*. (*h*) Tannin compounds gave results similar to MERCK'S tannin instead of "A."

13. A supernormal growth of aerial mycelium was usually accompanied by a corresponding reduction in pycnidia formation.

14. Harmful effects of tannin were also frequently shown by the absence of natural pigment from the mycelial pellicle and by the ashen color of the aerial hyphae. One or both of these might be present in the same series of cultures.

15. While tannic acid is no doubt toxic to many parasitic fungi, there are also other substances associated with tannin which are toxic. Some of these substances respond to the ordinary tannin tests and have probably been mistaken for tannin. The factors which enable plants to resist the attacks of parasitic organisms present an extremely complicated problem. The solution of this problem lies in the study of the chemistry and physiology of the cell.

16. Throughout the summary the terms "tannin" and "tannic acid" have been used in the generally accepted sense, but experiments with KERR'S extracts "1–X," "2–X," and "3–X" indicate that the toxic property is in the coloring material, which in analytical work is usually estimated as tannin.

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